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NO CROSSING OVER IN THE MALE OF DROSOPHILA OF GENES IN THE SECOND AND THIRD PAIRS OF CHROMOSOMES.

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In a brief note in *Science* for November 22, 1912, it was pointed out that there was no "crossing over" in the male between the factors for black body color and vestigial wings, while between the same factors in the female there was 22 per cent. of crossing over. The data then published have been extended and revised,[1] and the results can now be given in detail. For purposes of easy comparison I shall present the data in the same order as those given in the preliminary report.

It had been shown by Morgan and Lynch (BIOL. BULL., XXIII., 1912) that when black flies having normal (long) wings are mated to gray flies having vestigial wings, only three classes appear in F_2, viz.:

Gray Long.	Black Long.	Gray Vestigial.	Black Vestigial.
4569	2151	1626	0

It was pointed out later (*Science*, 1912) that the absence of the black vestigial class would be expected, if, in F_1, no interchange of factors occurs in one sex. The following analysis will make evident why, on the assumption that there is no crossing over in the gametogenesis of the F_1 male, there are no black vestigials expected in F_2.

[1] We have learned better how to control the conditions of culture so that less disturbance results from differential viability. Many of the old data, although consistent within themselves, are not strictly comparable with the recent data obtained under better conditions. For the case of black and vestigial the data presented here differ from those reported (*Science*, 1912) in that new data have been substituted for some of the old data in the tests of the amount of crossing over in the female.

Black long ♀, bV_g—bV_g.
Gray vestigial ♂, Bv_g—Bv_g.

F_1 ♂ or ♀, bV_gBv_g.

Gametes of F_1 { BV_g—bV_g—Bv_g—bv_g Eggs
bV_g—Bv_g Sperm.

F_2 {
BV_g bV_g, gray long, BV_g Bv_g, gray long.
bV_g bV_g, black long, bV_g Bv_g, gray long.
Bv_g bV_g, gray long, Bv_g Bv_g, gray vestigial.
bv_g bV_g, black long, bv_g Bv_g, gray vestigial.

The expectation is 4 gray long, 2 black long, 2 gray vestigial, 0 black vestigial. The data show that the gray vestigial run behind expectation, which is due to viability (crowding out through competition). It is evident that the expectation would be the same if the failure to cross over occurred in the female instead of in the male as here assumed. In order to test which sex failed to give crossing over the following matings were made. (1) Black, long winged females were mated to gray, vestigial winged males. The F_1 flies ♂ and ♀ were gray, long. The F_1 males were bred to black vestigial females and gave *two* classes.

Gray Long.	Black Long.	Gray Vestigial.	Black Vestigial.
0	19	19	0
0	30	33	0
0	34	20	0
0	193	115	0
0	174	118	0
0	542	416	0
0	992	721	0

If we assume that there has been no crossing over in the F_1 heterozygous males the result is explicable, as shown in the following analysis, except in so far as the gray vestigials are crowded out.

Black vestigial ♀, bv_g—bv_g,
F_1 ♂, bV_g—Bv_g,

F_2 bv_g bV_g, black long,
bv_g Bv_g, gray vestigial.

The F_1 heterozygous females (sisters to the males just tested) were also tested by crossing to black vestigial males. *Four* classes were produced.

Gray Long.	Black Long.	Gray Vestigial	Black Vestigial.
66	283	201	58
65	243	224	50
55	218	169	42
20	113	112	29
71	311	210	47
17	153	181	20
44	231	218	48
338	1,552	1,315	294

This result, in contrast to the last one, is explicable on the assumption that crossing over occurs in the female, as the following analysis shows:

F_1 ♀, BV_g—bV_g—Bv_g—bv_g,
Black vestigial ♂, bv_g— bv_g

BV_g bv_g, gray long.
bV_g bv_g, black long.
Bv_g bv_g, gray vestigial.
bv_g bv_g, black vestigial.

The expectation on the basis of *free* crossing over in the female is 2 gray long, 2 black long, 2 gray vestigial, 2 black vestigial. The two classes gray long and black vestigial are cross over classes, and their smaller numbers result from linkage. It will be noted that the two vestigial classes fall below the corresponding long classes, and this can be accounted for as due to viability. The percentage of crossing over on the basis of the data is 17.9.

These last two experiments show that the realized classes are explicable on the basis of non-crossing over of factors in the heterozygous male, and of crossing over to the extent of 17.9 per cent. in the female. This conclusion can be tested by bringing into the cross the same factors in other combinations. One such test is as follows. Gray, long winged females (BV_g) were bred to black, vestigial males (bv_g) and produced gray long males and females. The F_1 heterozygous males produced in this way were mated to black vestigial females, and gave:

Gray Long.	Black Long.	Gray Vestigial.	Black Vestigial.
18	0	0	18
90	0	0	95
174	0	0	78
102	0	0	86
384	0	0	277

This result is what is to be expected on the basis of no crossing over in the heterozygous male as the following analysis shows:

Black vestigial ♀, bv_g—bv_g
F_1 ♂, BV_g—bv_g

bv_g BV_g, gray long.
bv_g bv_g, black vestigial.

The F_1 heterozygous females (sisters to the males just tested) were in turn mated to black vestigial males and gave:

Gray Long.	Black Long.	Gray Vestigial.	Black Vestigial.
255	55	47	184
163	24	37	139
47	9	11	33
121	18	16	109
586	106	111	465

These results are explicable if crossing over (17.1 per cent.) takes place in the F_1 heterozygous females, as indicated below:

F_1, bV_g—BV_g—bv_g—Bv_g
Black vestigial, bv_g—bv_g

bV_g bv_g, black long.
BV_g bv_g, gray long.
bv_g bv_g, black vestigial.
Bv_g bv_g, gray vestigial.

It has been pointed out that when the factors for black and for long wings go in together, *i. e.*, enter with the same chromosome, and the factors for gray and for vestigial enter with the other chromosome (it does not matter which parent contributes each) the double recessive does not appear in F_2. In more general terms it may be said, for the second chromosome, that when two recessives enter, one from each side, the double recessive does not

appear in the F_2 generation. The explanation of this is clear on an examination of the first analysis. Since there is no crossing over in the male, each gamete in the male will contain one or the other of the dominant factors, hence no double recessive zygote can appear, and every zygote must contain at least one dominant. This means that there is one class with two dominants, one with the one, one with the other. If we assume that the factors are carried by specific chromosomes, and that the factors for black and for vestigial (and hence for their normal allelomorphs) lie in the homologous chromosomes, we can explain the results just given on the basis that no interchange of factors occurs between these chromosomes in the male even though in the female interchange takes place. Why an interchange should take place in one sex and not in the other cannot be stated, but I should not be surprised if a thorough cytological study would throw some light on the subject.

The converse case is that of two recessives entering from the same side, in which case all the expected classes appear in F_2. An example with its analysis will make this clearer. Gray, long winged females (double dominants) were mated to black vestigial males (double recessives) and gave gray long winged F_1 offspring. These F_1 flies, mated, gave in F_2:

Gray Long.	Black Long.	Gray Vestigial.	Black Vestigial.
246	9	18	63

The following analysis shows why the result to be expected here is different from that in the last case. Half of the gametes

Gray long ♀, BV_g—BV_g.
Black vestigial ♂, bv_g—bv_g.

F_1 ♂ and ♀, BV_g—bv_g.

Gametes of F_1 bV_g—BV_g—bv_g—Bv_g Eggs
BV_g—bv_g Sperm

bV_g BV_g, gray long, bV_g bv_g, black long,
BV_g BV_g, gray long, BV_g bv_g, gray long,
bv_g BV_g, gray long, bv_g bv_g, black vestigial,
Bv_g BV_g, gray long, Bv_g bv_g, gray vestigial.

in the male contain both recessive factors and in consequence, even with no crossing over in the male, the realization of a double recessive zygote becomes possible.

The expected ratio without linkage in the female is 5 : 1 : 1 : 1. Four classes are expected and four appear, but the ratio given above is entirely changed by the linkage of the factors which here, on our assumption, lie in the same chromosome.

Purple Eyes and Vestigial Wings.

An eye color called purple also shows linkage with vestigial and is placed, therefore, in the second group of factors. It has likewise been used to test non-crossing over in the male. Purple vestigial flies mated to red-eyed, long-winged flies gave red-eyed, long-winged F_1 males and females. When these F_1 males were bred to purple vestigial females only two classes were produced:

Red Long.	Purple Long.	Red Vestigial.	Purple Vestigial.
52	0	0	62
141	0	0	113
96	0	0	131
28	0	0	34
68	0	0	89
22	0	0	33
112	0	0	90
519	0	0	552

The result is explicable if no crossing over occurs in the male and the analysis is the same in principle as that for the black vestigial case. As explained before, every sperm contains one or the other recessive since they went in separately and have not crossed over in the male.

When the F_1 females (sisters to the males just tested) were mated to purple vestigial males, the following results were obtained:

Red Long.	Purple Long.	Red Vestigial.	Purple Vestigial.
2,876	270	270	2,433

The result is explicable on the hypothesis of crossing over (about ten per cent.) in the female, as the analysis for the comparable case for black vestigial shows.

Purple-eyed, long-winged flies were mated to red-eyed, vestigial flies, and gave normal F_1 flies. When the F_1 males were bred to purple vestigial females the following results were obtained:

Red Long.	Purple Long.	Red Vestigial.	Purple Vestigial.
0	62	42	0
0	78	70	0
0	61	53	0
0	66	103	0
0	79	90	0
0	346	358	0

Here again the results are explicable on the hypothesis of non-crossing over in the male. The recessives enter the F_1 male from opposite sides, *i. e.*, they lie in different but homologous chromosomes. Hence if no crossing over occurs in the male each spermatozoon will contain one or the other recessive, and since the eggs are all alike and carry only double recessive factors only two types of zygotes are expected.

When the F_1 females, sisters to the males just tested, were likewise bred to purple vestigial males, the following classes result:

Red Long.	Purple Long.	Red Vestigial.	Purple Vestigial.
265	2,203	2,038	234

Crossing over in the female (about 10 per cent.) explains the classes that appear in this experiment.

These results with purple vestigial are comparable at every point with those for black vestigial and both furnish evidence of no crossing over in the male. The numbers are large enough to make it highly probable that crossing over in the male did not occur.

Further Examples of Non-crossing over in the Male Between Genes in the Second Pair of Chromosomes.

The following data are taken from experiments that Mr. C. B. Bridges and the author are carrying out. The records have been made by Mr. Bridges. A recessive mutant called "dachs" (from the short legs of the flies) was combined with another character also in the second group, viz., black. When the double recessive dachs black is crossed to the wild (normal gray) fly

the offspring are normal. When an F_1 male was mated to dachs black females the following results were obtained:

Normal Gray.	Dachs Gray.	Normal Black.	Dachs Black.
98	0	0	72

These results are consistent with the hypothesis of no crossing over in the male. Since dachs lies on the other side of black from purple and vestigial, the data extend the application to a new section of the second chromosome, and considerably increase the significance of the results.

Another recessive mutant stock has wings curved in an arc. "Arc" was combined with black—both belonging to the second group of factors. When black arc was mated to wild flies (with straight wing) the offspring were normal. An F_1 male back crossed to black arc gave the following results:

Gray Straight.	Black Straight.	Gray Arc.	Black Arc.
161	0	0	145

This result also is conformable with the hypothesis of no crossing over in the male, and since arc lies on the same side of black as does purple and vestigial but considerably beyond either, still a new section is found to be free from crossing over in the male. Further, since in the female there is a very great amount of crossing over (about 36 per cent.) between black and arc, the lack of crossing over in the male is even more striking.

When black was crossed to purple the F_1 flies were gray red. An F_1 male was mated to a black purple female and gave in the next generation:

Gray Red.	Black Red.	Gray Purple.	Black Purple.
0	74	71	0

These results are again conformable with the hypothesis. In this case the two recessives entered from different sides.

Another mutant caaracter, streak, is a dominant. Streak gray mated to normal (thorax) black gave streak gray F_1 flies. An F_1 male was mated to the double recessive, normal black females, and gave the following classes:

Streak Gray.	Normal Gray.	Streak Black.	Normal Black.
19	0	0	21

Again the results conform. Here the two dominants entered from one side and two recessives from the other, and the double recessive normal black was used for testing the gametes of the F_1 male. Streak lies at the most extreme end of the chromosome so far as known, on the same side of black as does dachs, and on the other side from purple, vestigial and arc. The interval between streak and black is nearly as long as that between black and arc, so that the results are of interest because the whole section from streak on the one hand to arc on the other is known to give the same result, namely, a complete absence of crossing over. These two loci, streak and arc, are so far apart that in the female there is practically free Mendelian assortment.

No Crossing over in the Male between Genes in the Third Pair of Chromosomes.

A test of whether or not crossing over takes place in the male between genes in the third group; or, as we say, in the third chromosome, was first published for the case of pink and ebony by Sturtevant (*Science*, 1913), who showed that no crossing over occurred in the F_1 male for the small number of males tested. The following case gives similar results for another pair of loci. The data are taken from experiments that are being carried out by C. B. Bridges and the author.

A mutant stock has kidney-shaped eyes. A double recessive stock of pink kidney was made up, both factors being in the third chromosome. Pink kidney mated to wild flies gave F_1 flies with red round eyes. The F_1 males back crossed to pink kidney females gave the following results:

Red Round.	Pink Round.	Red Kidney.	Pink Kidney.
23	0	0	15
11	0	0	9
56	0	0	50
91	0	0	61
6	0	0	8
22	0	0	17
11	0	0	5
6	0	0	2
63	0	0	58
142	0	0	114
431	0	0	339

These results are in harmony with the hypothesis of no crossing over in the male between genes in the third group of chromosomes. The amount of crossing over between pink and kidney in the female is about 15 per cent., which is greater than the most frequent value of about 5 per cent. in Sturtevant's case of pink ebony.

Discussion.

In their paper of 1911, Bateson and Punnett described certain phenomena that they called coupling and repulsion. The numerical and class results offer many points of similarity to the cases here described. In a later paper they gave reasons for abandoning the earlier view of repulsion as distinct from coupling. In this paper (December, 1911) they gave two cases in which linkage occurs, but in which crossing over must be assumed to take place in both sexes. Bateson and Punnet postulate in order to account for their results that segregation takes place at some early stage in the germ-tract. Whatever form of interpretation may apply to the cases described by Bateson and Punnett, the tests that I have made of F_1 males and females show, that in *Drosophila* at least, the results are due to failure of crossing over between factors in one sex. It may be that in certain animals and plants crossing over is the same in both sexes, while in other cases it may be that crossing over is different in the two sexes. Whether this is true or not can only be determined by making tests like those here employed. Until such tests have been made for other forms in which linkage has been found we cannot know how widely the explanation here followed may be extended.

A NEW GENE IN THE SECOND CHROMOSOME OF DROSOPHILA AND SOME CONSIDERATIONS ON DIFFERENTIAL VIABILITY.

C. B. BRIDGES AND A. H. STURTEVANT.

Morgan and Lynch (BIOL. BULL., '12) and Morgan (*Science*, '12) have reported the linkage relations of two non-sex-linked genes, black and vestigial.[1] Morgan considered these two genes as lying in a "second chromosome," the first chromosome being the sex chromosome. He showed that in the female there was a considerable amount of crossing over between these two genes, but in the male there was none at all so far as the data showed.

At the time when this linkage between black and vestigial was first observed we were engaged in a systematic search for linkage between non-sex-linked genes in *Drosophila*. One of us (Bridges) had already observed in the F_2 generation of a cross of black by curved[2] that no black curved flies appeared. We interpreted this case as one of linkage of such a strong order that no crossing over had taken place. On the basis of this linkage we concluded that curved was in the same chromosome as black, that is, in a "second chromosome." The similar work of Morgan on black and vestigial showed that the non-appearance of the double recessive in a case in which two second chromosome recessives entered the F_1 from opposite parents, could be explained on the basis of lack of crossing over in the male. The present paper shows that the same explanation applies to the case of black by curved, and further deals with the determination of the amount of crossing over in the female between the black and the curved loci.

An individual heterozygous for two allelomorphic pairs as *AB*, *ab* may form four kinds of gametes, namely *AB*, *ab*, *Ab*, and

[1] Vestigial was at that time called wingless.

[2] "Curved" a wing mutant discovered by Bridges is characterized by the thin texture of the wings which are held out widely from the body and curved. This and other mutants are shortly to be described in detail by Morgan and Bridges.

aB. In case of linkage, however, the two new combinations (here *Ab* and *aB*) are not represented in as large numbers in the gametes as are the original combinations (*AB* and *ab*). The actual ratio in which these gametes are formed can be calculated only very indirectly from an F_2 zygotic ratio, and if the two F_1 individuals are forming the gametes in different ratios, the calculation may be impossible.

As suggested by Baur (*Verh. naturf. Ver. Brünn*, '11) what is needed is to test these double heterozygotes by an individual all of whose gametes are of one kind, namely, recessive for both factors in question (*aa*, *bb*) since in this case they will not mask any combination in the gametes tested. Baur's method, then, is to test double heterozygotes by double recessives. The proportion in which the zygotes appear is a direct measure of the gametic proportions. In such zygotic proportions there are two equal classes representing original combinations (in the example *AB* and *ab*) and two other equal classes representing recombinations or crossovers. In calculating the percentage of crossing over therefore, we add these cross over classes together and divide by the total number of zygotes. Since in the case of sex-linked characters the male producing sperm is analogous to a double recessive, the F_2 males will in effect always be such a back cross test.

Flies with curved wings from pure stocks were mated to stock black flies. The F_1 flies were gray not-curved, that is, like the wild fly in appearance. F_2 consisted of:

Wild Type.	Black.	Curved.	Black Curved.
391	194	168	0
458	226	165	0
Total 849	420	333	0

The absence of black curved in F_2 is the result to be expected if there is no crossing over in the male, no matter what the percentage of crossing over may be in the female. However, if there is crossing over in the female a few of the F_2 blacks should be heterozygous for curved and a few of the curved heterozygous for black. The most advantageous procedure to get the double recessive from these F_2 flies is to mate in mass cultures the blacks

of one sex to the curved of the other sex. In F_3 if there has been crossing over in the F_1 ♀ there should appear some of the double recessives or some single recessives heterozygous for the other single recessive. In fact some blacks appeared which gave, in F_4, 3 black : 1 black curved. From these black curved individuals a pure stock of the double recessive was obtained directly.

That the absence of black curved flies in F_2 was really due to lack of crossing over in the male was shown by making "back-cross" tests of doubly heterozygous males as follows:

Black × curved.
↓
F_1 ♂♂ × black curved ♀♀.
↓

Wild Type.	Black.	Curved.	Black Curved.
0	35	40	0
0	44	31	0
0	25	20	0
0	18	15	0
0	45	60	0
0	39	33	0
0	90	83	0
0	50	51	0
0	29	34	0
0	375	367	0

From the converse cross of wild by black curved described below, two tests of F_1 males were made, with the following results:

Wild × black curved.
↓
F_1 ♂♂ × black curved ♀♀.
↓

Wild Type.	Black.	Curved.	Black Curved.
108	0	0	88
71	0	0	57
179	0	0	145

No new combinations of characters (crossovers) appeared in the 1,066 flies from the two converse experiments. This is in exact agreement with Morgan's results on black vestigial, and is somewhat more significant, since, as will appear below, there

is in our case more crossing over in the females than in the combination studied by Morgan.

We have seen that when black and curved enter F_1 separately, one class, namely, the double recessive, does not appear in F_2, for the reason that a double recessive gamete is not formed in the F_1 male through lack of crossing over. But if black and curved enter from the same parent then half the gametes of the F_1 male are doubly recessive, and, therefore, give in a back cross test the amount of crossing over in the female. The other half of the gametes of the male are the double dominant class and in consequence half of the flies fall into a single double dominant class. If, then, there is crossing over in the female in the ratio of one crossover gamete to n of the original combination, the F_2 will consist of $n : 1 : 1 : n$ zygotes from the doubly recessive sperm, and a like total, that is $2n + 2$, from the doubly dominant half of the sperm. The F_2 proportions expected are therefore $3n + 2 : 1 : 1 : n$ and the ratio of each single recessive class (1) to the double recessive class (n) gives the gametic ratio directly. The results of such a cross as that described appear below:

Wild × black curved.

↓

F_2

	Wild Type.	Black.	Curved.	Black Curved.
	298	27	15	48
	88	7	5	8
s*[1]	252	21	15	56
s*	310	19	28	79
	948	74	63	191

The occurrence of black and of curved flies in this experiment demonstrates that crossing over takes place, and from the evidence of the preceding experiments, we conclude that it must have been in the females. That this is the correct interpretation is shown by direct tests of such females. As stated above, the percentage of crossing over can be calculated directly from tests

[1] In all counts reported here *s* signifies that the record includes only the offspring of a single female. For reasons which will appear in future publications from this laboratory it has seemed advisable to include only the first cultures obtained from any females. When later cultures are available but have been omitted, the record will be marked with an asterisk.

of this nature. In the following table the calculation for each culture and for the total is given.

Wild × black curved
↓
F_1 ♀ × black curved ♂♂.
↓

	Wild Type.	Black.	Curved.	Black Curved.	Per Cent. of Crossovers.
	96	31	21	96	21.3
s*	63	20	17	78	20.7
s*	103	34	40	102	26.5
s*	106	27	44	105	25.2
s*	112	34	55	127	27.2
s	130	38	49	144	24.1
	610	184	226	652	24.5

Similar tests of F_1 females from the converse cross of black × curved gave:

	Wild Type.	Black.	Curved.	Black Curved.	Per Cent. of Crossovers.
	88	203	212	80	28.8
	68	161	159	80	31.6
	56	221	256	69	20.8
	67	373	286	84	18.7
	70	252	240	64	21.4
s	17	97	95	26	18.3
s	27	113	92	21	19.0
s	33	152	141	28	17.2
s	18	100	99	27	18.4
s	11	50	56	8	15.2
s*	45	144	150	55	25.4
s*	41	134	119	52	27.1
s*	56	157	148	40	23.9
s*	47	135	95	29	24.8
	644	2,292	2,148	663	22.7

Adding the figures from these two converse experiments gives 1,717 crossovers in 7,419 flies, or 23.1 per cent. of crossovers. Because of double crossing over, which is known from unreported experiments to occur within this distance, 23.1 is slightly less than the actual chromosomal distance apart of these two loci.[1]

It will be seen from the two above tables that there is apparently a rather wide range of variability in the percentage of crossing over in different cultures. This variability is much greater than one would expect to find if it were due entirely to

[1] See Sturtevant (*Jour. Exp. Zool.*, '13) for a discussion of linear series of genes within a chromosome.

chance deviations; and one might therefore be led to suppose that it is due to an actual variation in the strength of linkage. While this conclusion may be correct, it is not necessary, since there is another important factor which must be considered—namely, the effects of differential viability. In order to get definite data regarding the manner of action of this disturbing element we have made some crosses in which it may be studied without the complication of linkage. If curved flies heterozygous for black ($B\ c_v\ b\ c_v$) be mated to blacks heterozygous for curved ($b\ C_v\ b\ c_v$), the same four classes of flies as in the above tables should be produced, but now in equal numbers. If equality is not shown the deviation cannot be due to linkage, but must probably be attributed either to the error of random sampling, or to differential viability. The results actually obtained in these experiments are shown in the following table:

Black[1] (het. for c_v) × curved (het. for b).

	Wild Type.	Black.	Curved.	Black Curved.
I.s.....	31	31	22	29
II.....	46	48	40	48
III.....	35	27	30	14
IV.s.....	44	53	33	65
V.s.....	14	31	18	22
VI.....	52	50	56	41
VII.....	47	31	24	36
VIII.s.....	43	31	2	20
IX.s.....	13	7	10	15
X.s.....	37	43	43	28
XI.s.....	37	51	36	26
XII.s.....	14	14	14	17
XIII.s.....	76	78	56	46
	489	495	384	407

That there really is no linkage present is evident from the totals, since the complementary classes give totals of 879 and 896, respectively. The deviation of these numbers from equality is less than half the standard error—a very close approximation to expectation. These totals show some effects of differential viability, especially in that the curved flies were less numerous than those with normal wings. A study of the individual bottles brings out some other interesting points. It is obvious that

[1] The first five cultures below were from curve ♀ × black ♂, the others from the reciprocal cross (black ♀ × curved ♂).

viability may not always produce the same kind of effect. For instance, in culture VIII., the curved class ran far behind all three others, yet in VI. it was the largest class of all. The same relations are shown by the black curved class in XIII. and IV. respectively, and in several other cases. It also appears that the viability difference between two classes differing in two characters is not always merely a summation of the effects produced by these characters separately. Thus in culture VII., since the curved and the black class are each behind the normal class, we might expect the class which is both black and curved to be still further behind—yet it is really ahead of both single recessive classes. In culture VIII. black is slightly behind normal, but black curved is far ahead of curved (gray). It is obvious from these considerations that it is not possible to work out "coefficients of viability" and use them for making corrections in our data, since with respect to viability the deviations are not constant in amount or direction. However, it is to be noted that when conditions are made as favorable as possible the error from viability is reduced considerably, and often becomes very slight indeed. There is evidence which indicates that differential viability is often due to unequal sensitiveness to starvation, dryness, or similar unfavorable conditions. Several of the cultures recorded in the last table above were purposely kept under various poor conditions (small bottles, little food, etc.,) in order to test this point. Cultures II., III., and VIII. are examples showing the results produced, and III. and VIII. are among the most aberrant cultures in the table. The remedy, then, would seem to be in choosing mutants which are of nearly the same vigor as the normal, and in keeping the cultures in good condition—plenty of room and good food.

Even under these conditions there may be a high mortality, but that this need not always be a *differential* mortality is indicated by an experiment which we have carried out. Three females from the cross of black by curved were tested by backcrossing to black curved males. The eggs were counted daily, and the offspring produced were recorded, with the following result:

Wild Type.	Black.	Curved.	Black Curved.	Total.	Total No. Eggs.
34	91	96	32	253	550

Thus although less than half the eggs produced flies, there is no evidence of differential variability, since the complementary classes are approximately equal, as expected.

COLUMBIA UNIVERSITY,
January, 1914.

THE INFLUENCE OF THE ENVIRONMENT ON THE SIZE OF EXPECTED CLASSES.

T. H. MORGAN AND SABRA COLBY TICE.

In crosses in which rudimentary wings are involved, it has been apparent, since this race was first bred, that the classes containing rudimentary wings often run far behind expectation. The experiments made clear that the character rudimentary wings is a Mendelian recessive and is sex-linked. The deficiencies that appeared were assigned to viability of these flies. We have found meanwhile for other stocks that by breeding *pairs* of flies in large bottles, with an abundance of food, kept in good condition, there was a very marked increase in number of those classes that are deficient in number if many flies are bred in small bottles, or even in large bottles if so many parents are used that crowding of the larvae takes place. It was determined, therefore, to repeat the experiments with rudimentary wings under the most favorable conditions that our experience had made known to us. In order to avoid the possible criticism that the stock might have changed, a control culture *en masse* was again made in which crowding took place.

Results similar to these with rudimentary wings had also come up in crosses in which a new mutant, "strap wing" was involved. This factor is not sex linked, but belongs to our second group. Similar experiments were carried out with this stock.

The Viability of the Rudimentary Winged Race.

In a paper in *Science*, 1911,[1] an F_2 count is given in which 5,850 long-winged flies (♂ and ♀) and 83 rudimentary-winged males are recorded. The expectation is that of these 5,850 flies one third should be long-winged males, or 1,950. This number is also the expectation for the rudimentary-winged males. Instead of 1,950 there are only 83 males or 1/23 the expected number.

[1] *Science*, Vol. XXXIII., March 31, 1911.

The F_1 generation of the reciprocal cross was published in *Science*, 1912.[1] The rudimentary-winged female bred to a long-winged male gave 381 long-winged daughters and only 3 rudimentary-winged sons where equality was expected. Whether the lack of sons here is due entirely to viability, or to other conditions as well cannot be stated.

In a very brief paper in 1911[2] some other data were given that showed the rudimentary classes running behind expectation. These data were corrected and expanded in another paper,[3] to which reference may now be made. An F_2 count is given there, that is an extension of the data published in the first paper referred to above. There are 14,309 long-winged grandchildren (♂♂ and ♀♀) and 115 males with rudimentary wings. The expectation here is that one third of 14,309 or 4,769 flies should have rudimentary wings. The entire number is 115, or only 1/41 of the expected number.

There is an F_1 count of the reciprocal cross when 68 long-winged daughters and 3 rudimentary-winged sons appear. This ratio is not lower than that given above, and may safely be ascribed to viability. In the F_2 count of the same combination the following classes and numbers were realized:

Long ♀	721
Long ♂	698
Rud. ♀	163
Rud. ♂	237

The expectation is for equal numbers. The rudimentary males, while far behind expectation, are not so far behind as in other crosses cited, which is due to better treatment. This same statement applies to the remaining data of the 1912 paper for, at that time realizing more fully the influence of the environment on viability larger bottles with more food were used. Since most of the data involves miniature wings it is not cited here.

In the new experiments rudimentary-winged males were bred to long-winged (wild) females; and long-winged daughters and sons obtained. The daughters were then back-crossed to rudimentary males either in pairs or *en masse*. When bred *en masse*

[1] *Science*, Vol. XXXIV., March 22, 1912.

[2] *Proc. Soc. Exp. Biol. and Medicine*, VIII., February, 1911.

[3] *Zeit. f. induktive Abstammungs und Vererbunglehre*, VII., 1912.

the following offspring were obtained. The expectation is for equality in all four classes:

Long ♀	527
Long ♂	489
Rudimentary ♀	7
Rudimentary ♂	31

There is an enormous deficit in the classes of rudimentary flies. Instead of equality there are only 1/27 as many as expected.

When on the other hand the F_1 females were back-crossed *in pairs* the following totals were obtained:

Long ♀	1,717
Long ♂	1,545
Rudimentary ♀	1,120
Rudimentary ♂	1,179

There is an approach to equality in the last case (1 : .7), and, in consequence, the contrast with the preceding data is striking. For purposes of more detailed comparison the data for the two mass cultures and for the 20 pairs is given:

TABLE I.

BACK-CROSS PAIRS.

No.	Long ♀.	Long ♂.	Rudimentary ♀.	Rudimentary ♂.
7	133	117	94	102
8	59	43	55	47
9	107	114	67	66
10	70	55	46	64
11	62	60	53	36
12	49	36	51	41
13	86	76	31	43
14	135	101	61	104
15	107	102	61	79
16	67	44	60	54
17	116	107	73	70
18	92	80	72	68
19	87	77	84	53
20	16	14	12	6
21	78	101	73	72
22	83	60	44	50
23	88	81	62	91
24	52	49	35	37
25	95	100	56	65
26	135	128	30	31
Total.....	1,717	1,545	1,120	1,179

BACK-CROSS EN MASSE.

28	248	235	4	14
29	279	254	3	17
Total.....	527	489	7	31

This same mating was again made both in mass and in pairs. The expectation is equality of long and rudimentary wings. The mass cultures gave:

Long ♀	341
Long ♂	337
Rudimentary ♀	51
Rudimentary ♂	64

When the experiment was made with pairs the following totals were obtained, (with a ratio of 1. : .63):

Long ♀	1,676
Long ♂	1,431
Rudimentary ♀	930
Rudimentary ♂	1,022

For detailed comparison the counts of the two mass cultures and of the 29 pairs taken separately are given in Table II.

TABLE II.

BACK-CROSS PAIRS.

No.	Long ♀.	Long ♂.	Rudimentary ♀.	Rudimentary ♂.
31	161	122	108	154
32	15	7	5	4
33	154	121	27	30
34	82	103	38	66
35	103	81	11	13
36	143	74	12	42
37	86	84	62	49
38	59	79	51	57
39	123	98	67	61
40	164	142	70	85
41	63	59	71	54
42	72	45	47	42
43	56	65	55	55
44	47	51	47	46
45	45	37	34	25
46	93	64	42	47
47	45	50	46	33
48	63	47	44	55
49	59	45	52	65
50	63	57	41	39
Total.....	1,676	1,431	930	1,022

BACK-CROSS EN MASSE.

51	154	135	18	19
52	187	202	33	45
Total.....	341	337	51	64

Since rudimentary females are sterile with rudimentary males the stock of rudimentary wings is maintained by breeding rudimentary males to heterozygous, long-winged females.

THE VIABILITY OF THE STRAP-WING RACE.

The peculiarities of structure and inheritance of this mutant will be described in another place. The stock contained "beading" and overlaps in appearance the vestigial wing. The "beaded" flies that appear in F_2 are classified with "normal" or long wing, those with vestigial-like wings are counted in with "strap." Strap bred to wild stock gives sons and daughters with long wings. These bred *en masse* during August gave the following results in eleven cultures. About six to ten F_1 flies were used in each culture in a medium sized bottle, and the food conditions were made as favorable as possible.

	Normal.	Strap.	Ratio.
1.	224	59	1 : 3.8
2.	236	63	1 : 3.7
3.	471 (+39 curved)	28	1 : 17.0
4.	216	45	1 : 4.8
5.	103	11	1 : 9.3
6.	277	24	1 : 11.5
7.	162	14	1 : 11.6
8.	464	23	1 : 20.1
9.	494	82	1 : 6.2
10.	288	44	1 : 6.5
11.	164	34	1 : 4.8
Total.....	3,099 (+39)	427	1 : 7.2

The ratios range from 1 : 3.7, to 1 : 20.1. In order to compare these results with matings in pairs, fifteen pairs were mated. After ten days the parents were placed in a new bottle with fresh food and in some cases they were carried to a third bottle. The results are given in the next table (III.).

The results from pairs approximate more nearly to expectation (1 : 3); the totals give a ratio of 1 : 3.57. The individual lots

TABLE III.

F_2 FROM STRAP ♂ × WILD ♀.

No.	Brood I.					Brood II.					Brood III.				
	Normal.		Strap.		Ratio to 1.	Normal.		Strap.		Ratio to 1.	Normal.		Strap.		Ratio to 1.
	♀	♂	♀	♂		♀	♂	♀	♂		♀	♂	♀	♂	
1	97	89	27	17	4.2	218	199	60	47	3.9	—	—	—	—	—
2	112	116	24	20	5.2	148	141	23	26	5.9	48	44	16	14	3.1
3	90	76	13	12	6.6	88	88	8	5	13.5	—	—	—	—	—
4	117	101	37	14	4.3	99	130	38	28	3.5	25	41	10	16	2.4
5	73	63	24	31	2.5	80	88	34	30	2.6	7	3	1	1	—
6	79	90	23	26	3.5	119	100	30	24	4.1	10	14	2	5	—
7	105	86	31	27	3.3	121	115	27	37	3.7	50	59	12	23	3.1
8	94	91	32	22	3.4	120	113	39	36	3.1	24	37	10	6	3.8
9	95	87	19	31	3.6	101	106	29	29	3.6	59	42	17	27	2.3
10	72	60	31	26	2.3	46	59	22	20	2.5	5	—	1	—	—
11	66	63	12	22	3.8	57	36	5	7	7.7	—	—	—	—	—
12	79	68	27	38	2.3	77	77	28	24	3.0	3	3	1	—	—
13	79	65	26	15	3.5	54	62	17	18	3.3	37	29	10	5	4.4
14	85	80	28	26	3.1	78	90	22	21	3.9	32	34	11	7	3.8
15	62	61	14	15	4.1	63	53	21	26	2.5	30	17	7	7	3.4
	1,305	1,196	368	342	3.52	1,479	1,457	403	378	3.75	330	323	98	111	3.12

are worthy of inspection. The ratios run more evenly and range from 1 : 2.3 to 1 : 7.7 with one exceptionally high at 1 : 13.5. The counts from day to day (not recorded here) show that the F_2 strap-winged flies hatch later than the flies with normal wings, so that as the cultures run out, the relative number of strap-wing flies increases, and unless the cultures are exhausted when the count stops there will be a deficiency of strap-wing flies. An attempt was made to run the cultures to a finish, although this cannot always be done. If the cultures dry up, a disproportionately large number of strap-winged flies will be destroyed, and this will account in part for the deficiency in this class. These results are not affected by the age of the parents, as shown by comparison of the ratios in the totals for the second (1 : 3.75) and third (1 : 3.12) broods with that of the first (1 : 3.52).

The above pairs were mated in November and December, 1913. In order to make a more exact comparison, a few mass cultures were made in December from the same stocks. The results are shown in the next table (IV.).

In addition to the preceding data there were other crosses of strap male by wild female that had been made nine months

TABLE IV.

No.	Normal.	Strap.	Ratio.
1	455	67	1 : 6.8
2	365	65	1 : 5.6
3	330	75	1 : 4.4
Total.....	1.150	207	1 : 5.5

before those recorded above. In these earlier experiments in mass cultures no attempt was made to avoid crowding through use of too many parents, although the bottles were otherwise maintained in good condition. The following F_2 counts were made:

	Long ♀ and ♂.	Strap ♀ and ♂.	Ratio.
1.	739	76	1 : 9.7
2.	411	55	1 : 7.5
3.	247	22	1 : 11.2
4.	410	59	1 : 6.9
5.	464	54	1 : 8.6
6.	266	33	1 : 8.0
7.	490	61	1 : 7.8
8.	342	43	1 : 8.1
9.	137	13	1 : 10.5
10.	493	3	1 : 164.0
11.	245	8	1 : 30.6
12.	197	0	———
13.	818	137	1 : 6.0
14.	614	125	1 : 4.9
Total.....	5.873	689	1 : 8.53

Whether the two very high ratios under 10 and 12 should be included may be questioned. It is possible though not probable that contamination took place. Excluding these the ratios vary from 1 : 4.9 to 1 : 30.6.

CONCLUSIONS.

The experiments show that under unfavorable conditions due to crowding the mutant forms, rudimentary wings and strap wings, run behind expectation. That the result is due to crowding is shown when cultures of sister individuals are made *in pairs* with abundant room and food. There is then a closer, and in some cultures a complete agreement with expectation. In experiments in which it is only necessary to show whether a character is recessive or dominant, and whether it is, or is not

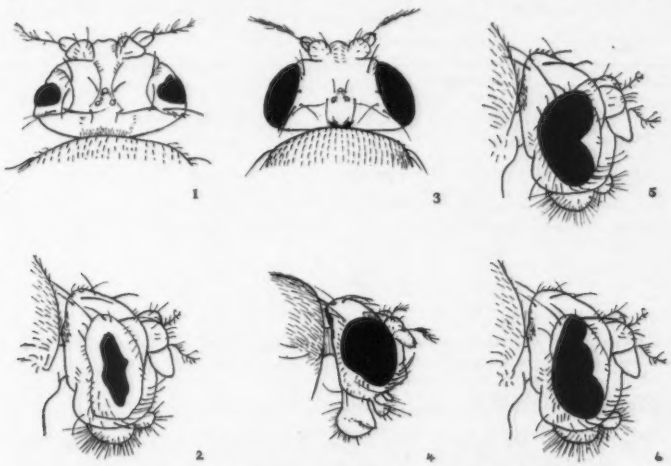
linked to other characters these deficient ratios, while unfortunate, present no serious difficulties, but in experiments in which it is necessary to determine accurately the linkage ratios the difference becomes serious.

The rudimentary and strap-winged mutants have been used to test the effects of crowding due to mass breeding, because they have been found to show such effects more than any other stocks. In the case of other mutants that give, even in mass cultures, nearly the theoretical values, the method which we now generally employ of breeding by pairs in large culture bottles with plenty of food eliminates almost entirely disturbances due to viability.

A NEW SEX-LINKED CHARACTER IN DROSOPHILA.

SABRA COLBY TICE.

A new sex-linked character (Figs. 1 and 2) recently appeared in *Drosophila ampelophila* in an experiment involving rudimentary and long-winged flies with normally shaped eyes (Figs. 3, 4). The new character "barred" eye appeared in a single



FIGS. 1 AND 2. Pure barred (male) above and from the side.
FIGS. 3 AND 4. Normal eye (side view smaller fly).
FIGS. 5 AND 6. Two heterozygous eyes, both in side view.

male. The ommatidia are reduced in number and are restricted to an area shaped like a vertical bar or band, the edges of which are more or less irregular in shape.

BARRED EYE BY NORMAL EYE.

When the barred male was bred to normal-eyed females, the F_1 generation showed the barred eye in the females only (Figs. 5 and 6), the males being normal. This is what would be expected if the new character is sex-linked and dominant. The

F_2 generation (Table I.) with class ratios 1 : 1 : 1 : 1 is consistent with this expectation. In the following analyses Br' is the factor for the dominant barring and br' is its recessive normal allelomorph.

TABLE I.

P_1 Normal ♀, $X\ br' - X\ br'$.
Barred ♂, $X\ Br' - ——$.

F_1 Females. $\begin{cases} X\ br' \\ X\ Br' \end{cases}$ Barred ♀.	F_1 Males. $\begin{cases} X\ br' \\ —— \end{cases}$ Normal ♂.
570	565

Gametes of F_1 barred ♀, $X\ br' — X\ Br'$.
Gametes of F_1 normal ♂, $X\ br' — ——$.

	F_2 Females.		F_2 Males.	
	$\begin{cases} X\ br' \\ X\ br' \end{cases}$	$\begin{cases} X\ Br' \\ X\ br' \end{cases}$	$\begin{cases} X\ br' \\ —— \end{cases}$	$\begin{cases} X\ Br' \\ —— \end{cases}$
No.	Normal ♀.	Barred ♀.	Normal ♂.	Barred ♂.
2	170	136	158	122
3	160	140	142	140
4	155	124	154	119
5	92	105	91	79
6	81	58	87	63
7	112	88	82	83
8	102	86	87	93
9	104	84	92	89
10	87	72	84	90
Total.....	1,063	893	977	878

The barred females of the above F_2 generation were heterozygous in barring, and, when mated to their barred brothers, gave an equal number of barred and normal sons (Table II.).

TABLE II.

Gametes of F_2 barred ♀, $X\ Br' — X\ br'$.
Gametes of F_2 barred ♂, $X\ Br' — ——$.

F_3 Females. $\begin{cases} X\ Br' \\ X\ Br' \end{cases}$ $\begin{cases} X\ br' \\ X\ Br' \end{cases}$ Barred ♀.	F_3 Males. $\begin{cases} X\ Br' \\ —— \end{cases}$ Barred ♂.	$\begin{cases} X\ br' \\ —— \end{cases}$ Normal ♂.
236	106	123

The original barred male was crossed to his daughters (F_1 ♀ ♀, Table I.) and as expected the results (Table III.) were similar to those of Table II.

TABLE III.

Gametes of F_1 barred ♀, *X br'—X Br'*.
Gametes of P_1 barred ♂, *X Br'— ——*.

Females.		Males.	
X Br'	*X br'*	*X br'*	*X Br'*
X Br'	*X Br'*	——	——
Barred ♀.		Normal ♂.	Barred ♂.
281		159	140

In the experiments recorded in Tables II. and III. it was noticed that the barring was of two kinds: (1) A very narrow bar (Figs. 1 and 2) which appeared in all barred males, and in about one half of the double class of barred females, and (2)

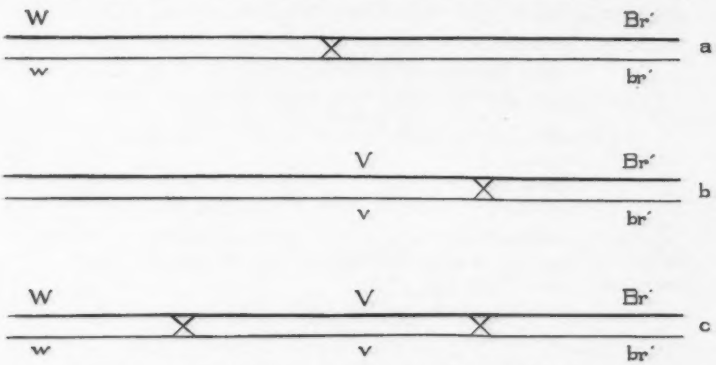


DIAGRAM 1. (*a*) Showing loci of white (*w*) and barred (*br'*) in sex chromosomes of heterozygous female, with the loci of their normal allelomorphs. Crossing over is indicated by the *X* between the lines. (*b*) Ditto for vermilion and barred. (*c*) Ditto for white, vermilion, and barred.

a bar not so narrow which was present in the rest of the females. The loss in the latter case was mainly on the anterior edge and was especially noticeable as a single notch, or two notches, giving the eye a decided heart shape (Figs. 5 and 6). Here also the ommatidia are disturbed from their regular geometrical arrangement, and converge in rows to the notch. The difference in the narrow and broad bars is due to a difference in zygotic composition for, as will be described later, breeding tests have shown that invariably the broader types are heterozygous in barring (*Br' br'*). When the narrow bar females were bred to

barred males all the offspring were narrow barred. This stock has since bred true.

Red-barred Eye by White Normal Eye.

The original barred male was crossed with white-eyed females, with the results given in Table IV. The F_1 ♀ ♀ were red barred, and the F_1 ♂ ♂ were white normal, since white is a sex-linked character. The F_2 generation consisted of four classes, red barred, white barred, red normal, white normal, with an equality of males and females in each. The ratios of the four classes will be discussed later. In the following analyses, *w* is the factor for the recessive white (sex-linked) and *W* is its normal allelomorph (red).

Table IV.

P_1 White normal ♀, *X w br′*—*X w br′*.
Red-barred ♂, *X W Br′*— ——.

F_1 Females.	F_1 Males.
X w br′ *X W Br′*	*X w br′* ——
Red Barred ♀.	White Normal ♂.
483	440

Gametes of F_1 barred ♀ *X w br′*—*X W Br′*—*X w Br′*—*X W br′*.
Gametes of F_1 white ♂—*X w br′*. – ——

	F_2 Females.				F_2 Males.			
	X w br′, *X w br′*,	*X W Br′*, *X w br′*.	*X w Br′*, *X w br′*.	*X W br′*, *X w br′*.	*X w br′*, ——	*X W Br′*, ——	*X w Br′*, ——	*X W br′*, ——
No.	White Normal ♀	Red Barred ♀.	White Barred ♀.	Red Normal ♀.	White Normal ♂.	Red Barred ♂.	White Barred ♂.	Red Normal ♂.
101	72	84	31	43	86	75	46	71
102	74	58	41	53	65	60	55	52
103	62	81	40	47	57	66	43	52
104	45	35	29	36	21	41	28	30
105	46	58	30	42	35	48	30	46
106	26	44	18	21	24	27	20	29
107	32	44	23	22	25	32	15	19
108	35	43	27	32	31	58	33	45
109	34	41	25	36	31	38	27	39
Total...	426	488	264	332	375	445	297	383

The white heterozygous barred females (F_2 above), bred to their white barred brothers, gave the expected proportions (Table V.).

TABLE V.

Gametes of F_2 white barred ♀, *X w Br'*—*X w br'*.
Gametes of F_2 white barred ♂, *X w Br'*— ——.

No.	F_2 Females.	F_2 Males.	
	$\{X\ w\ Br', \{X\ w\ br', \{X\ w\ Br', \{X\ w\ Br'$	$\{X\ w\ Br', \{$ ——	$\{X\ w\ br', \{$ ——
	White Barred ♀ ♀.	White Barred ♂.	White Normal ♂.
a.	198	118	105
b.	224	116	101
Total	422	234	206

By mating together white narrow barred females (homozygous), and white barred males from the above experiment, a white barred stock was obtained which has since bred true.

The original mutant male was mated to his daughters (F_1, Table IV.) and gave the expected proportions (Table VI.).

TABLE VI.

Gametes of F_1 red barred ♀, *X w br'*—*X W Br'*—*X W br'*—*X w Br'*.
Gametes of P_1 red barred ♂, *X W Br'* — ——

Females.	Males.			
Red Barred ♀.	Red Barred ♂.	White Normal ♂.	White Barred ♂.	Red Normal ♂.
240	63	52	50	43

RED BARRED EYE BY VERMILION NORMAL EYE.

The original barred male was mated to vermilion eyed females with the results given in Table VII. As in the two previous cases the barred character was dominant and sex-linked, appearing in the F_1 generation in the females only. The F_1 females, as expected, were red and the males were vermilion, since vermilion also is a sex-linked character. The F_2 generation showed the expected sex-linkage results with four classes: vermilion barred, red normal, vermilion normal, and red barred, with an equality of males and females in each class. The ratios of these four classes will be discussed later.

In the following analyses *v* is the factor for the recessive sex-linked character vermilion and *V* is its normal allelomorph (red).

TABLE VII.

P1 Vermilion normal ♀, *X v br′*—*X v br′*.
Red barred ♂, *X V Br′*— ——.

	F1 Females.	F1 Males.
	X v br′	*X v br′*
	X V Br′	———
	Red Barred ♀.	Vermilion Normal ♂.
	367	312

Gametes of F1 red barred ♀, *X v br′*—*X V Br′*—*X v Br′*—*X V br′*.
Gametes of F1 vermilion normal ♂, *X v br′*— ——.

	F2 Females.				F2 Males.			
	X v br′, *X v br′*,	*X V Br′*, *X v br′*.	*X v Br′*, *X v br′*.	*X V br′*, *X v br′*.	*X v br′*, ——	*X V Br′*, ——	*X v Br′*, ——	*X V br′*, ——
	Vermilion Normal ♀.	Red Barred ♀.	Vermilion Barred ♀.	Red Normal ♀.	Vermilion Normal ♂.	Red Barred ♂.	Vermilion Barred ♂.	Red Normal ♂.
201	315	229	70	95	248	202	80	88
202	284	270	90	102	313	234	82	92
203	250	247	79	103	271	222	68	68
204	93	89	35	24	95	83	34	33
205	99	111	32	38	110	99	45	40
206	72	100	31	31	81	98	20	22
207	108	97	40	57	112	76	43	56
208	141	102	37	46	97	105	36	28
209	125	108	26	28	98	88	26	26
Total...	1,487	1,353	440	524	1,425	1,207	434	453

The heterozygous vermilion barred females (F_2 above) were mated to their vermilion barred brothers and gave the expected ratios (Table VIII.).

TABLE VIII.

Gametes of F2 vermilion barred ♀, *X v Br′*—*X v br′*.
Gametes of F2 vermilion barred ♂, *X v Br′*— ——.

	F2 Females.	F2 Males.	
	X v Br′, *X v Br′*. *X v Br′*, *X v br′*.	*X v Br′*, ———	*X v br′*, ———
	Vermilion Barred ♀.	Vermilion Barred ♂.	Vermilion Normal ♂.
a.	243	131	124
b.	278	125	169
Total...	521	256	293

By mating vermilion narrow barred females (homozygous)

to their vermilion barred brothers, from the above experiment, vermilion barred stock was obtained which has since bred true.

The original male was mated to his daughters (F_1 Table VII.) and gave the expected proportions (Table IX.):

TABLE IX.

Gametes of F_1 red barred ♀, *X v br'*—*X v Br'*—*X V Br'*—*X V br'*.
Gametes of P_1 red barred ♂, *X V Br'*— ——.

Females.		Males.		
Red Barred ♀.	Red Barred ♂.	Vermilion Normal ♂.	Vermilion Barred ♂.	Red Normal ♂.
124	37	48	18	18

THE LINKAGE OF THE FACTOR FOR BARRED EYE.

Since the character barred is sex-linked it follows the sex chromosomes. It remains to determine its linkage with other sex-linked factors. Red barred males bred to white normal females gave in the F_2 generation (see Table IV.) the following results:

	White Normal.	Red Barred.	White Barred.	Red Normal.
♀	426	488	264	332
♂	375	445	297	383
Total.....	801	933	561	715

The non-cross over classes are red barred and white normal. The cross over classes are white barred and red normal (Diag. 1*a*). These two categories gives 42.8 per cent. crossing over.

In order to determine the linkage of barred to another sex-linked factor, viz. vermilion; red barred males were bred to vermilion normal females. The F_2 generation (see Table VII.) gave the following results:

	Vermilion Normal.	Red Barred.	Vermilion Barred.	Red Normal.
♀	1,487	1,353	440	524
♂	1,425	1,207	434	453
Total.....	2,912	2,560	874	977

The percentage of crossing over in the above case is 25.3 per cent. Diag. 1*b*.

Earlier experiments (Sturtevant, '13) have shown that there is about 30.7 per cent. of crossing over between white and vermilion. Since there is 25.3 per cent. crossing over between vermilion and barred the factor for barred must lie close to white (approximately 5.4) or else very far from white. That the latter is the case is shown by the large percentage of crossing over between white and barred (42.8 per cent.).

Other work done in this laboratory, largely as yet unpublished, shows that, when distances as long as this between white and barred are involved, the chromosomes often break at two points and re-unite. As a result of this double-crossing over, the non-cross over classes are increased and the cross over classes are diminished (Diag. 1*c*). The amount by which the percentage 42.8 is less than the percentage 30.7 + 25.3 is therefore an indication of the amount of double crossing over that has occurred. Sturtevant ('13) has located certain factors on the *X*-chromosome. Vermilion (*B*) is given as 30.7. The experiments in this paper giving 25.3 per cent. crossing over between vermilion and barred enable us to locate the new factor barred approximately at 56 (30.7 + 25.3) (without considering the double crossing-over between white and vermilion or that between vermilion and barred).

Dominance.

In these experiments whenever flies were heterozygous for barring they showed the barring without exception. In this sense the dominance is *constant*, in that a fly which fails to show the barring cannot transmit it. As was stated before the homozygous females have a narrower bar than the heterozygous females. That it is possible to pick out at sight the two different flies is verified by the following experiments.

P1 Broad Barred ♀ (Heterozygous) by Barred ♂.

F_1	Barred ♀.	Barred ♂.	Normal ♂.
46	80	31	52
47	119	71	72
49	138	80	81
50	101	50	65
51	101	50	56
52	127	75	63
62	94	55	55
65	107	62	48
Total.....	867	464	492

P1 Narrow Barred ♀ (Homozygous) by Barred ♂.

F_1.	Barred ♀.	Barred ♂.
90	65	60
91	43	35
56	86	76
58	101	117
60	118	119
64	90	94
Total.....	503	501

P1 White Broad Barred ♀ (Heterozygous) by White Barred ♂.

F_1.	White Barred ♀.	White Barred ♂.	White Normal ♂.
146	62	30	27
147	86	36	39
151	88	37	44
152	69	31	23
Total.....	305	134	133

P1 White Narrow Barred ♀ (Homozygous) by White Barred ♂.

F_1.	White Barred ♀.	White Barred ♂.
148	81	72
149	83	78
150	45	51
153	105	95
154	63	60
157	38	47
Total.....	415	403

P1 Vermilion Broad Barred ♀ (Heterozygous) by Vermilion Barred ♂.

F_1	Vermilion Barred ♀.	Vermilion Barred ♂.	Vermilion Normal ♂.
249	57	23	29
256	84	39	42
257	67	45	36
258	87	39	37
Total.....	295	146	144

P_1 VERMILION NARROW BARRED ♀ (HOMOZYGOUS) BY VERMILION BARRED ♂.

F_1	Vermilion Barred ♀.	Vermilion Barred ♂.
246	67	72
247	30	39
248	52	46
250	42	37
253	43	50
254	48	46
Total.....	282	290

VIABILITY.

In the foregoing experiment where an equality of barred and normal was expected, it is evident that the mutant character is almost as viable as the normal. For this reason it should prove valuable for further work on linkage. In the experiments in which no linkage is involved, there are 4,671 barred among 9,578 flies. The percentage of barred is 48.7 where 50 is expected. Where the experiments involving linkage are included, the barred flies total 9,767 out of 20,240 and the percentage is 48.2. The relative viability of white and barred appears in those experiments in which both were raised under the same conditions as 45.4 per cent. to 49.9 per cent. The relative viability of vermilion and barred was 51.7 per cent. to 46.8 per cent.

CONCLUSION.

Barred eye—a new sex-linked character—appeared in *Drosophila* in a single red-eyed male fly and its dominance is constant over the normal eye. The broad bar of the heterozygous females distinguishes them from the homozygous ones with a narrow bar. The viability of the stock makes it valuable for linkage experiments.

By taking the percentage of cross overs as an indication of distance on the chromosome, we can place the factor for barred approximately at 56.

ZOÖLOGICAL LABORATORY COLUMBIA UNIVERSITY,
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ANOTHER CASE OF MULTIPLE ALLELOMORPHS IN DROSOPHILA.

T. H. MORGAN.

It has been shown[1] that the factor for white eye color and the factor for eosin eye color are allelomorphs. Each arose as a mutation, but unlike other original and independent mutations they do not give, when crossed, the wild type (red eye) by recombination. Each factor has the same normal allelomorph in the wild fly, and each factor gives the same linkage ratio as the other one with any sex-linked factor. The factor for eosin, the factor for white and their normal allelomorph form a system of triple allelomorphs. Only two of these factors can exist at any one time in the same female, and, since they are sex-linked, only one at a time in a male. In consequence, a red-eyed female may be heterozygous for white or for eosin, but never for white and for eosin.

Another similar case has also appeared. In certain crosses, involving yellow body color, a yellowish male appeared that was characterized by a light spot on the upper surface of the posterior end of the abdomen (Figs. 3 and 4). When the yellow stock was examined another male of the same kind was found there. It is evident that in the first case the new type must have come from the original yellow stock. The mutation probably occurred in the stock one or more generations before it was discovered.

It was natural to assume that such a peculiar spot could be transferred to gray or to black flies. The original male and later many of his offspring that bore the same character were mated to gray and to black flies. The first generation was always normal gray. In the second generation the yellows and the yellow blacks ("browns") were spotted, but never any of the gray or the black flies. Thinking that the spotting was closely linked to the yellow factor and might cross over if enough flies were bred I continued the experiment for four or five months.

[1] Morgan and Bridges, *Journ. Exp. Zool.*, XV., 1913.

While no record of the number of flies that were examined was kept, I think more than 30,000 gray and black flies must have been looked over. In no case was the spot present.

The alternatives then are absolute (or possibly nearly absolute) linkage, or a case of multiple allelomorphism. In practice these alternatives would give the same numerical results. The theoretical differences between the idea of absolute linkage and multiple allelomorphism will be discussed later.

When a spot male was mated to wild females, all the offspring were normal gray without spot. When the F_1 offspring were inbred the following classes were produced.

	Gray ♀.	Gray ♂.	Spot ♂.
a.	285	132	94
b.	305	127	113
Total.....	590	259	207

There is one class of females and two classes of males. This is the expectation if spot is due to a sex-linked factor. It is the same kind of result that obtains when a yellow male is crossed to a wild female. When an F_1 female from the last experiment was back crossed to a spot male the following results were obtained:

	Gray ♀.	Spot ♀.	Gray ♂.	Spot ♂.
a.	100	88	91	84
b.	25	16	15	20
c.	86	87	70	48
Total.....	211	191	176	152

Equality in all four classes is expected and this is practically realized. The spot type seems somewhat less viable than the wild type. The male counts run behind the female, which is a usual result. Here the spot also occurs in the female (Figs. 7 and 8).

A spot female was mated to a yellow male. The 63 daughters were yellow without spot which shows that spot is completely recessive to yellow. The 58 sons were spot, receiving their sex chromosome from the mother. When a pair of these F_1 flies were bred, the following F_2 classes were produced.

Yellow ♀.	Spot ♀.	Yellow ♂.	Spot ♂.
44	45	41	45

This is the expected result for this combination.

The body-color black belongs to our second group of linked factors. A spot female was mated to a black male. The daughters were gray without trace of spot, the sons were spot. Female producing sperms bring in the normal allelomorph for spot (Y), which, with the normal allelomorph for black (B), gives gray. The sons are spot because the male receives his sex chromosome from his mother. The formulas for the F_1 and F_2 generations are the following:

Spot ♀, $y_s B$ — $y_s B$.
Black ♂, Yb — — b.

F_1 { Gray ♀, $y_s B\ Yb$.
Spot ♂, $y_s B$ — b.

$y_s B$ — Yb — $y_s b$ — YB, Eggs of } F_1.
$y_s B$ — $y_s b$ — — B — — b, Sperm of }

$y_s B y_s B$, spot.	$y_s B$ — B, spot.
$Yb y_s B$, gray.	Yb — B, gray.
$y_s b y_s B$, spot.	$y_s b$ — B, spot.
$YB y_s B$, gray.	YB — B, gray.
$y_s B y_s b$, spot.	$y_s B$ — b, spot.
$Yb y_s B$, black.	Yb — b, black.
$y_s b y_s b$, spot black.	$y_s b$ — b, spot black.
$YB y_s b$, gray.	YB — b, gray.

The F_2 expectation is 3 gray, 3 spot, 1 black, 1 spot black, and is the same for the male and the female classes which are counted together in the following F_2 results for this cross.

F_2 Females.		F_2 Males.	
Gray.	Spot.	Black.	Spot Black.
133	90	47	39

The double recessive is spot black, which corresponds in color to yellow black or "brown" of earlier papers. The light spot shows here even more strikingly, because of contrast, than in the simple "spot" itself.

Pure stock of the double recessive spot black being on hand, a male was crossed to a yellow female. The F_1 were yellow flies, 65 males and 20 females without spot, showing again the dominance of yellow to spot in the "compound" y-y_s. These F_1 flies inbred gave:

Yellow ♀.	Yellow Black ♀.	Yellow ♂.	Yellow Black ♂.	Spot Black ♂.	Spot ♂.
126	37	33	26	11	26

A male of the spot black stock was also bred to a black female. The offspring were black males and females, which inbred in four lots gave the following results:

	Black ♀.	Black ♂.	Spot Black ♂.
1.	313	165	109
2.	291	140	75
3.	396	211	85
4.	494	255	144
5.	363	178	115
Totals.....	1,857	949	528

In these mass cultures, although an attempt was made to have the food conditions favorable, yet, owing to crowding, the spot black males ran far behind the black males.

There appeared in the above F_2 a yellow black fly with no trace of spot. Since in some cultures (but not here) the spot may be faint and difficult to detect with certainty, it seemed possible that the fly was such a *somatic* variant. He was tested by breeding to a black female. The offspring were black (very few in number). These inbred gave:

	Black ♀.	Black ♂.	Yellow Black ♂.
1	55	19	32
2	316	172	151
3	59	42	29
Total.....	430	233	212

Had the male in question been only a somatic condition of yellow (suppressed spot) we should have expected some spot males in the F_2 generation. Since none at all appeared there can be no doubt that the grandparent was in reality a *yellow* black individual both somatically and gametically. The most prob-

able explanation is that a mutation backwards from spot to yellow took place (with no change in the black). Another interpretation is that contamination had occurred. This seems highly improbable since at the time there was no stock of yellow black in the laboratory, and only a few experiments under way in which such individuals were being produced. Nevertheless, so long as there were no other characters than these in the experiment by means of which the offspring could be further identified, I think that one cannot be too careful in interpreting such rare occurrences as mutation backwards. A third possibility is that a cross over occurred, and if so, spot is closely linked to yellow, and crossed over only in this one case out of the many thousands of cases.

In order to see whether the spot would show in the female of the "compound" yellow-spot, when the compound was also black, a spot black female was bred to a yellow black male. All the daughters were yellow black without trace of spot. The sons were spot black. These mated together gave the following F_2 generation.

	Yellow Black ♀.	Spot Black ♀.	Yellow Black ♂.	Spot Black ♂.
1.	15	29	18	25
2.	45	47	42	25
3.	60	62	61	51
Totals.....	120	138	121	101

Other Crosses with Spot.

We have another stock of black-colored flies called ebony which belongs to our third group of factors. When spot female was crossed to ebony male the daughters were gray and the sons were spot. These inbred gave in F_2 the following results.

Gray.	Spot.	Ebony.	Spot Ebony.
91	102	29	24
106	73	45	28

The analysis is the same as in the case of spot by black. The double recessive is spot ebony which has a brownish color differing in tint from the brown of spot black.

There is still another black-colored mutant called sable. It is

sex-linked, and belongs, therefore, in our first (or sex-linked) group of factors. When a spot female was bred to sable male the daughters were gray and the sons spot. Inbred these gave the following results.

Gray ♀.	Spot ♀.	Spot ♂.	Sable ♂.	Gray ♂.	Spot Sable ♂.
35	30	17	20	24	8

The analysis is the same as when the cross is made with yellow, except that gray is formed by recombination here. The linkage of spot and sable modifies the ratios somewhat.

In the cross with ebony and in that with sable there appeared in the F_1 generation a few exceptional cases that are due to non-disjunction. The actual results are

	Spot ♀.	Gray ♀.	Spot ♂.	Gray ♂
F_1	0	112	104	3
	0	66	52	4
	Spot ♀.	Gray ♀.	Spot ♂.	Sable ♂.
F_1	2	62	64	2

These cases are like those described by Bridges and call for no further comment here. They are explicable on the assumption that the two sex chromosomes of certain spot females occasionally stick together, and either pass out of the egg or else both remain in it. In the first work done more than a year ago with spot, some unexplained results were obtained but not then interpreted. It now appears that in part at least they may have been due to non-disjunction.

The Origin of Multiple Allelomorphs.

It is noteworthy that spot appeared in yellow stock. It may seem that there is some causal connection here since spot is a mutation in the same locus as yellow. Likewise eosin appeared in white stock, and is another case of multiple allelomorphism. Plausible as is the assumption it can not be proven, but the following considerations are not without interest. If the mutation appeared first in the yellow stock, in a chromosome that went into a male, the spot would be apparent on examination. If the chromosome went into a female the mutation would not appear for another generation and then in a male. If on the other hand the mutation had appeared in a chromosome in the gray stock

it might appear in the next generation if it went into a male, just as yellow itself might have appeared in the same way. If the chromosome in question had passed into a female no evidence of the mutation would be seen, but it would appear in the second generation males. The two cases are alike. Since, however, the mutation has never appeared in the gray stock, although a great many more gray flies have been seen than yellow flies, it might be argued that a mutation in a locus is more likely to be followed by another in the same locus than if no mutation had taken place there. But mutations are such rare phenomena that this argument does not carry much weight, and judgment must be suspended until further mutations have shown themselves.

Other Characteristics of Spot.

In general we are apt to seize upon the most evident characteristic of a mutation, give it a name, and neglect to mention, or overlook, other effects that may be associated with it. In the case of the white eye mutation for instance the eye attracts our attention, yet if red-eyed and white-eyed flies be put into alcohol the yellow, especially the yellow bands, become markedly different in the two flies. The white-eyed flies show much whiter bands. Evidently the mutation has affected other pigments than those contained in the eye.

In another mutation called club wing (as yet undescribed) the wing pads of many flies fail to expand, although others do expand even in pure stock. It was later found that a small bristle on each side of the thorax is absent in the club stock irrespective of whether the wing pads expand or not. This microscopic change is a constant feature of the mutation and a better character for that reason than the more obvious although inconstant one of the club-shaped wing.

Likewise in "spot." In the black spot fly there is a distinct light dot on the post thorax, and often a light band down the middle of the thorax (Fig. 4a). These were called dot and dash, but it was later noticed by Bridges that the dot and dash are present in flies only for a short time and disappear as the flies get older. All attempts to get stocks with permanent dot and dash

failed, because, as we now think, these are only juvenile characters. The dot is sometimes seen in young spot flies, but is weak and soon disappears. Dot and dash are, therefore, due to the same mutation that gave the spot, but are ephemeral and are especially seen in the double recessive condition—black spot. The different degrees of viability shown by the different mutations may also be looked upon as effects associated with the mutation—effects of the highest importance in determining the chances of survival of the mutants, but of small value as indicators of the mutation itself.

Linkage of Spot and White.

If, as the evidence shows, spot is an allelomorph of yellow, and both have a common normal allelomorph, it was not considered worth while to carry out any extensive experiments in linkage, since the data for yellow would apply to spot. Incidentally, however, a few experiments were made with white-eyed flies, and may be given here.

A few yellow white males were bred to spot red-eyed females. The F_1 flies were yellow red females and spot red males. These inbred gave:

	Yellow Red ♀.	Spot Red ♀.	Yel. White ♂.	Spot Red ♂.	Yel. Red ♂.	Spot White ♂.
(1)	63	19	40	31	0	1
(2)	94	21	47	60	1	0

The linkage of spot and white shown by these figures is 1.11. That already determined for yellow white is 1.12. The coincidence is greater than might be expected with small numbers for loci so near together.

In another experiment a spot black, red-eyed male was mated to a yellow white-eyed female. The daughters were yellow red, the sons yellow white flies. These inbred gave:

Females.

Yellow Red.	Yellow Black Red.	Yellow White.	Yellow Black White.
45	11	41	9

Males.

Spot Red.	Spot-black Red.	Yellow White.	Yellow-black White.
39	13	33	12

The analysis follows:

Yellow white ♀, *ywBywB*.
Spot black ♂, y_s*Wbb*.

ywB— *ywB*, Eggs of
y_s*Wb*— —*b*, Sperm of } P_1.

F_1 ♀, *ywB*y_s*Wb*.
F_1 ♂, *ywB*—*b*,

Eggs *yWb*—y_s*WB*—*yWB*—*ywB*—y_s*Wb*—y_s*wb*—*ywb*—y_s*wB*,
Sperm ——— *ywB*—*ywb*— ——*B*— ——*b*, ———

F_2 Females.		F_2 Males.	
yWbywB,	yellow red.	*yWb* —*B*,	yellow red.
y_s*WBywB*,	yellow red.	y_s*WB*—*B*,	spot red.
yWBywB,	yellow red.	*yWB* —*B*,	yellow red.
ywBywB,	yellow white.	*ywB* —*B*,	yellow white.
y_s*WbywB*,	yellow red.	y_s*Wb* —*B*,	spot red.
y_s*wbywB*,	yellow white.	y_s*wb* —*B*,	spot white.
ywbywB,	yellow white.	*ywb* —*B*,	yellow white.
y_s*wBywB*,	yellow white.	y_s*wB* —*B*,	spot white.
yWbywb,	yellow black red.	*yWb* —*b*,	yellow black red.
y_s*WBywb*,	yellow red.	y_s*WB*—*b*,	spot red.
yWBywb,	yellow red.	*yWB* —*b*,	yellow red.
ywBywb,	yellow white.	*ywB* —*b*,	yellow white.
y_s*Wbywb*,	yellow black red.	y_s*Wb* —*b*,	spot black red.
y_s*wbywb*,	yellow black white.	y_s*wb* —*b*,	spot black white.
ywbywb,	yellow black white.	*ywb* —*b*,	yellow black white.
y_s*wBywb*,	yellow white.	y_s*wB* —*b*,	spot white.

As shown by the preceding analysis, the expectation, without considering the strong linkage between spot and white, is as follows:

Females.		Males.	
Yellow red	6	Yellow red	3
Yellow white	6	Yellow white	3
Yellow-black red	2	Yellow-black red	1
Yellow-black white	2	Yellow-black white	1
		Spot red	3
		Spot white	3
		Spot-black red	1
		Spot-black white	1

The four classes of females were realized in the results, but only four of the possible eight classes of males appeared. These four are the non-cross-over classes between spot and white; those absent are the crossovers. Considering the small numbers involved, the absence of these four classes of males is a probable event.

EXPLANATION OF PLATE I.

FIG. 1. Normal abdomen of male, dorsal view.
FIG. 2. Ditto, side view.
FIG. 3. Spot abdomen of male, dorsal view.
FIG. 4. Ditto, side view.
FIG. 4a. Thorax (dorsal view) of young spot black showing dot and dash.

1

3

4a

2

4

EXPLANATION OF PLATE II.

FIG. 5. Normal abdomen of female, dorsal view.
FIG. 6. Ditto, side view.
FIG. 7. Spot abdomen, dorsal view.
FIG. 8. Ditto, side view.

5

7

6

8

EXPLANATION OF PLATE III.

FIG. 9. Spot abdomen of male showing slight development of spot, dorsal view.

FIG. 10. Ditto, side view.

FIG. 11. Ditto, female, dorsal view.

FIG. 12. Ditto, side view.

9

11

10

12